

MICROGEOGRAPHIC VARIATION AS THERMAL ACCLIMATION IN AN INTERTIDAL MOLLUSC

EARL SEGAL^{1, 2}

Department of Biology, Kansas State Teachers College, Emporia, Kansas

A growing body of literature suggests that many poikilotherms are able to regulate, to a remarkable degree, their physiological activity rates. Regardless of the latitude over which certain species are distributed or the seasonal temperature change to which they are subjected, their physiological rates converge towards a mean value. To accomplish this relative constancy, northern populations and winter forms often have higher rates of activity, metabolism and development than southern populations and summer forms when measured at the same temperature. Animals thermally conditioned in the laboratory have also shown this compensatory phenomenon.

Ample documentation is provided in the comprehensive review of Bullock (1955), who also presents data showing temperature adaptation at the tissue, cellular and enzyme level, and in that of Prosser (1955), who summarizes evidence of compensatory adjustment to oxygen tension, osmotic pressure and drugs, as well as to temperature. Roberts (1952) and Dehnel (1955), who themselves have contributed studies of this problem, give additional references.

In 1953, Segal, Rao and James extended the known cases of intraspecific physiological differentiation with respect to temperature to include microgeographically separated individuals of the species. The heart rate in the limpet *Acmaca limatula* and water propulsion in the mussel *Mytilus californianus* were faster in samples from low intertidal levels than from high levels at any given temperature.

In the present study, an attempt has been made to corroborate and extend the initial findings on *A. limatula* and to ascertain whether the significant parameter of the difference in microhabitats is temperature. Besides heart rate, differences in gonad size and spawning readiness have been investigated. A major portion of this study constitutes an attempt to test the hypothesis that we are dealing with individual adaptations to habitat temperature.

MATERIALS AND METHODS

Habitat

The aspidobranch gastropod *Acmaca limatula* Carpenter is a eurytopic intertidal species which at Palos Verdes, California (Lat. 33° 43' N., Long. 118° 16' W.) has a vertical distribution of approximately 1½ meters from a mid-tidal to a low-tidal level. Highest and lowest individuals may be separated by as much as 20 meters

¹ The work was performed in the Department of Zoology, University of California, Los Angeles, Calif.

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of sloping, rocky beach; the nature of the beach prohibits interchange between higher and lower levels.

At each of four collecting sites (referred to in the text as sites 1, 2, 3, and 4) the low-level specimens were taken from below zero datum (mean lower low water: U. S. Coast and Geodetic Survey Tide Tables, Pacific Coast) where they are at the temperature of the surf but for a few hours each month. The higher level specimens were taken 1 to $1\frac{1}{2}$ meters above zero datum where they are subject to exposure about 50% of the time.

Collection and care of animals

Animals were removed from the substrate with a thin spatula and transported wet to the laboratory in enamel or plastic trays. In the laboratory the animals were covered with fresh sea water, aerated, and refrigerated at temperatures approximating the average ocean temperature for that season (see Fig. 7). Each day the water was replaced with fresh sea water at the same temperature.

During the winter and spring months an attempt was made to approximate the natural exposure time for high-level individuals. The water was poured off and the animals allowed to warm to room temperature and stand for 5–6 daylight hours.

Heart exposure

No later than 24 hours after collection, the heart was exposed by cutting a hole in the shell to the left and slightly posterior to the shell apex with a fine toothed trephine. This tool was designed to take different sized cutting heads: 3 mm. in diameter for cutting small shells, $4\frac{1}{2}$ mm. in diameter for cutting larger shells. A retractable pin in the center of the trephine prevented the cutting edge from wandering. The surface of the mantle exposed by the hole was flushed clean of shell particles with a fine stream of sea water.

Each animal was numbered with colored lacquer, then placed in a 10-inch finger bowl (15 to 20 individuals per bowl) which contained 1– $1\frac{1}{2}$ inches of sea water. The animals were returned to the refrigerator for one–two days to allow the gut to empty and permit recovery of the animals from any possible operative shock.

Recording procedure

The day heart beats were counted, two finger bowls were placed on a wire mesh platform two inches below the surface in a 15-gallon aquarium. Water circulated through the aquarium at a constant temperature $\pm 0.2^\circ$ C.

Initially, the water bath was at the refrigerator temperature and was then gradually lowered to 4° , 7° , or 9° C. Two to four hours were allowed for the animals to reach the lower temperatures. Temperatures were raised by increments of 5° C. to a maximum of 29° C. Animals were allowed two hours to reach each temperature.

Using a stop-watch, the number of seconds required for 10 heart beats was counted by eye. At lower temperatures a reading was taken of each animal in the group and this was repeated a second and third time. At higher temperatures, because of the greater possibility of error due to the increased heart rate, the read-

ings were taken 5 times. Plotted points (see Figs. 1, 3, and 4), are the average of these data converted to beats per minute.

The basic measurement used in this study is stable and reliable. A few minutes after the operation, which does not break the mantle, the heart rate settles to a value which is consistent over many hours and even days. Those animals showing excessive locomotor activity or irregularity of heart activity were discarded.

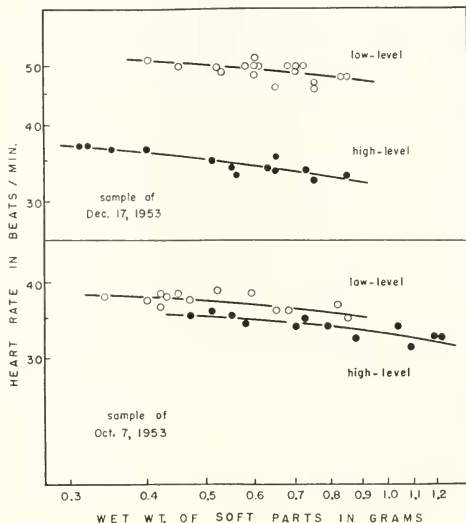


FIGURE 1. Relation between heart rate and wet weight of soft parts at 14°C . for vertically separated *A. limatula* from Palos Verdes. Points are averages of 3 to 5 readings of the number of seconds per 10 beats for one individual. The December collection shows a near maximum excursion of the difference in heart rate between samples. The October collection shows the minimum difference. Co-ordinates are logarithmic; equal percentage deviation is, therefore, shown by equal spatial spread from the hand-drawn regression lines anywhere on the graph. All curves are eye-fitted.

RESULTS

Effect of Intertidal Height on Heart Rate

When the animals' heart rates are measured at temperatures from 4° – 29°C ., it is found that the lower in the range of distribution an animal lives the faster is its heart rate. A comparison is made of the heart rates of "highs" and "lows" and the near maximum and the minimum difference in rate between samples are illustrated in Figure 1. At lower temperatures the absolute difference in heart frequency between "highs" and "lows" is less than it is at higher temperatures.

When heart rate is plotted against temperature, it can be seen that the curve for a low-level animal is above that for an equal weight individual from high-level

(Fig. 2). Since heart frequency will be shown to vary with habitat and season, rates at successive collections could not be combined. The pair of rate/temperature curves in Figure 2 have been selected from the many pairs calculated because they show the response both over the greatest range of temperatures and the largest number of temperature points.

When both groups are tested at any temperature, within the physiological ranges of temperatures of the species, heart rates of low-level animals are as much as 30 to 40% faster than those of equal weight animals from high-level. Heart rates of equal value for "highs" and "lows" are obtained when the high-level individuals are

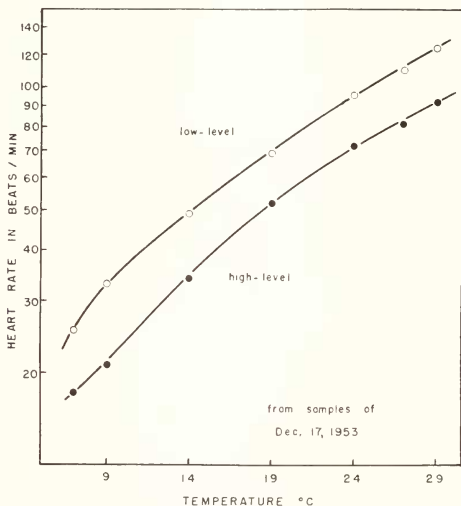


FIGURE 2. Heart rate as a function of temperature for equal weight (0.6 gm.) *A. limatula* from December collection of high- and low-level samples. Points represent intersection of perpendicular, erected at 0.6 gm., with weight regression curves at each temperature.

measured at temperatures 1° – $5\frac{1}{2}^{\circ}$ C. above that of low-level individuals. In February, for example (not graphed), a low-level individual of average weight (0.6 gm.) has a heart rate of 53 beats per minute at 14° C. An average high-level individual of equal weight shows the same rate when measured at 19.5° C.

In addition to the difference in position of the rate-temperature curves, there appears to be a reliable difference in the slopes as measured by the Q_{10} . Between 9° and 19° C., perhaps even above 19° C., the Q_{10} of the heart rate is consistently lower for low-level animals during the winter and spring months (Table I).

It would be of value to be able to state conclusively whether high-level individuals of the species are living at a warmer temperature than low-level individuals, at least within the local coastal area. An attempt will be made to establish this point.

High-level individuals are submerged approximately 50% of the time. During the hours of exposure these individuals are subjected to air temperatures which fluctuate about the prevailing water temperatures. A series of readings (taken with a thermistor probe, one mm. in diameter, inserted under the foot of limpets in place in the field) on a sunny day in late October show that body temperatures

TABLE I

Q₁₀ values of the heart rate for 0.6 gm. high- and low-level A. limatula over the temperature ranges indicated. Values calculated for equal weight animals on the weight regression curves at each collection period

Date	Temp. ° C.	Q ₁₀	
		High-level	Low-level
7/27/53	9-14	3.40	3.14
	14-19	2.56	2.53
	19-24	1.99	1.88
10/7/53	9-14	3.22	3.10
	14-19	2.38	2.89
	19-24	1.73	1.77
12/17/53	9-14	2.62	2.30
	14-19	2.34	1.98
	19-24	2.02	1.97
1/4/54	9-14	2.70	2.49
	14-19	2.18	2.16
	19-24	1.80	1.62
2/1/54	9-14	2.57	2.34
	14-19	2.54	1.97
	19-24	1.77	1.80
2/15/54	9-14	2.67	2.54
	14-19	2.38	2.05
	19-24	1.83	1.95
4/6/54	9-14	2.74	2.37
	14-19	2.02	1.78
	19-24	1.59	1.73
5/8/54	9-14	2.51	2.37
	14-19	2.11	2.02
	19-24	1.76	1.65

reached a high of 30° C. in the sun and 21.5° C. in the shade. Body temperatures may have reached higher values since the animals were exposed for an additional two hours. Over a 20-year period (U. S. Weather Bureau, personal communication) 40 to 50% of the days, from October through April, have been sunny at the local beaches. From November through April the surface water temperature averages slightly over 14.5° C.

Reference to Figure 8 shows that the annual range of the inshore surface water temperatures averages approximately 7°C . Air temperatures, taken a few feet above ground, show daily, and therefore monthly, fluctuations exceeding the yearly temperature range of the inshore waters. Of course, air temperatures a few feet above ground give only a rough directional estimate of microhabitat temperatures one cm. above ground. Over a period of $1\frac{1}{2}$ years, dry bulb recordings have shown the microhabitat temperatures to be consistently higher than prevailing air temperatures.

Minimum air temperatures falling below ocean temperatures are encountered primarily from late spring to early autumn when low tides generally expose high-level animals during late evening and early morning hours. During the part of this period when the ocean temperature is above 17°C ., the difference in heart rate between high- and low-level animals is at a minimum. The critical exposure occurs during winter and early spring in the late morning and afternoon hours. During this period the difference in heart rate is maximal.

It is of considerable interest, in this regard, that high-level animals show lower Q_{10} 's of the heart rate between 7° and 9°C . than low-level animals (low-level = 3.55, 3.66; high-level = 2.00, 2.15—two experimental recordings). It suggests that low-level animals are approaching cold depression at a higher temperature than are the high-level animals. The physiological temperature range is therefore believed to be wider for the relatively warm adapted high-level animals.

Influence of Certain Variables on Heart Rate

Body size. Among the numerous factors bearing influence upon physiological rate functions, size has been found to contribute to the variation in heart rate in the species under investigation. Size has been measured by the wet weight of soft parts. Within the weight range of 0.3 to 1.2 gm., larger animals show consistently slower rates at all temperatures from 4° to 29°C .

The regression of rate with weight is without apparent systematic variation over the year and is not significantly different between "highs" and "lows" at 14°C . ($P = .35$ for the difference between mean regression coefficients of 11 high-level and 12 low-level samples). Ten of the 23 samples show no reliable difference in regression from 9° – 24°C . The remaining 13 samples have larger negative b values on either side of 14° and 19°C . Hence, no single expression is available to describe fully the effect of weight on heart rate in this species.

Within the weight range of 0.4 to 1.0 gm., the regression is usually linear when plotted on logarithmic coordinates and varies from -0.043 to -0.172 . However, it is non-linear on either side of this weight range. It is as if we were plotting only a segment of a large parabola (see Figs. 1 and 3).

Both the factors of size and individual variation contribute to the scatter about each regression line. Size, however, is the major factor producing the scatter. Since weight and rate are inversely related, rate differences are meaningful only with essentially equal weight animals.

Sex. Sexes in *A. limatula*, as in all species of the genus, are separate, but three rather than two sexual states are present. To the conventional male and female is added the condition of indeterminacy. The latter is simply the post-spawning phase of the male or female in which gametes are absent (see section on gonad size).

It has not been possible to find a differential effect upon the heart rate that can be attributed to any of the sexual states excluding the pre-spawning animals heavy with gonads (see below).

Gonad size. An analysis of possible reproductive patterns will be presented in a following section. It is obvious from the data that the size and condition of the gonads vary over the year and between vertically separated individuals. Size of the gonad as such has a negligible effect on the heart rate; the condition of the gonads is, however, of importance.

On occasion when as many as 50% of a sample were possessed of insignificant gonads or were of indeterminate sex, and the remainder showed gonads weighing up to 20% of the body weight, the heart frequency of all animals fell within the scatter of either group. On the other hand, pre-spawning buildup of gonadal tissue, regardless of the size attained, rendered the heart beat erratic and not reliably measureable under our conditions. This was found in July for both high- and low-level individuals. In the latter part of the same month, when comparing heart rates of samples having approximately equal gonads by weight, high-level individuals showed slower rates consistent with the difference between groups throughout the year.

Diurnal rhythms. Two groups of 5 animals each were maintained at 14° C. and the heart beat counted at one-hour intervals over a 20-hour period. The animals were under constant illumination. Under these conditions, the presence of a day-night rhythm could not be demonstrated among either the high- or low-level individuals.

Effect of Transplantation on Heart Rate

Twenty-nine days. During March, 1953, 42 high-level and 42 low-level limpets were reciprocally transposed at site 1. Fifty control specimens from each level were handled in like manner but returned to their natural positions (see section on behavioral response). The numbers of recoveries are presented below.

Individuals transposed from low-level to high	52%
Individuals transposed from high-level to low	57%
Control individuals from high-level	84%
Control individuals from low-level	60%

Twenty-nine days after reciprocal transplantation, the heart rate, when measured from 4°–29° C., appeared to have undergone a complete reversal. Figure 3 shows the heart rate response of the transplants and controls at three selected temperatures. Heart rates of high-level individuals introduced into the low-level tide pool show a remarkable degree of overlap with those of the tide pool controls. An exception may be noted in the case of three individuals above 1.00 gm. in weight. However, since the rates for these animals fall within the variation of all transplants about the regression line, no significance has been attached to them.

Tide pool individuals transposed to high-level show heart rates close to but consistently faster than those of the high-level controls. Since animals transposed to the high-level position moved from their sites of placement to more protected positions (see below), the migrants have acclimated to a temperature somewhat lower than the "living" temperature of high-level controls.

Fourteen days. During January, 1954, a second reciprocal transplantation was

performed at site 4. Recovery was exceptionally poor. Of 50 high-level individuals transposed to low-level and 50 low-level controls, all but 2 and 5, respectively, were lost. Of the same number of low-level individuals transposed to high-level, 20% were recovered as compared with 50% of the high-level controls. Heart rates of low-level controls at the end of the experimental period and high-level transplants to low-level were therefore not available for comparison in significant numbers.

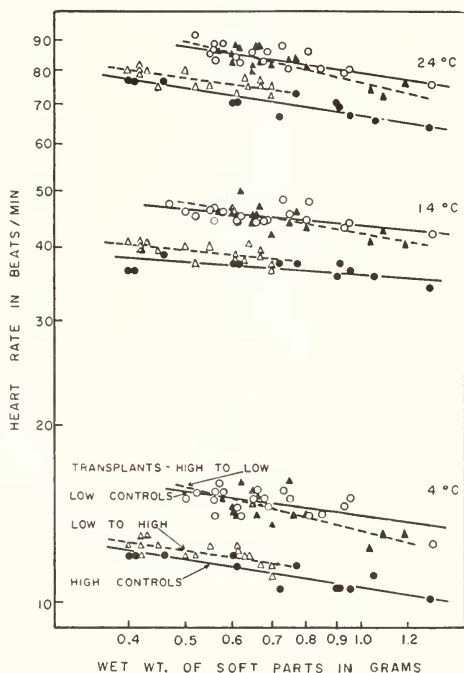


FIGURE 3. Relation between heart rate and wet weight of soft parts of reciprocal transplants and controls 29 days after transplantation. Each point represents one individual.

Comparing animals of equal weight, it is evident that the transplants to high-level have a lower heart frequency than initial low-level controls and a higher frequency than high-level controls at any temperature from 9°–24° C. (Fig. 4). The degree of acclimation of the transplants cannot be stated in equivalent °C., since the thermal history in the field cannot be given in simple terms. We only know that partial acclimation has occurred.

If we calculate the change in heart frequency of the transplants as a percentage of the difference in the frequencies of the low- and high-level controls, at the begin-

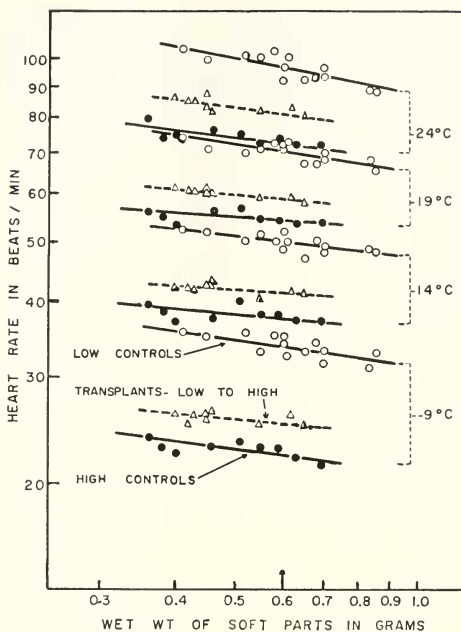


FIGURE 4. Relation between heart rate and wet weight of soft parts for second experimental transplantation. Duration of experiment 14 days. Each point represents one individual. Low-level controls measured at start of experimental period; high-level controls measured at end.

TABLE II

Calculation of per cent acclimation after transplantation from low- to high-level. Heart rate values are from equal weight (0.6 gm.) high- and low-level controls at start of experiment and high controls and transplants to high-level after 14 days. Rate values are taken from Figure 4. Per cent acclimation of transplants calculated from the difference in rate of low- and high-level controls at beginning and end of experiment. Performed in January 1954.

Temp. ° C.	Mean heart rates of average 0.6 gm. animals in beats/min.				% acclimation
	High controls 12/17/54	Low controls 12/17/54	Transpl. to high 1/4/54	High controls 1/4/54	
9	21.0	33.0	25.0	22.5	76.0
14	34.0	49.0	41.0	37.0	66.5
19	52.0	69.0	59.0	54.0	66.5
24	73.0	97.0	81.0	72.5	65.5
29	92.0	126.0	106.0	95.0	64.5

ning and end of the experimental period, we can roughly compare the response after 14 days with that after 29 days (Table II). At the 5 temperatures shown, an average of 68% of the difference in heart frequency between the groups has been achieved by the transplants in 14 days. However, since the transplants are acclimating to a temperature lower than that of the high-level habitat (see section on behavioral response), 68% may be too low a figure.

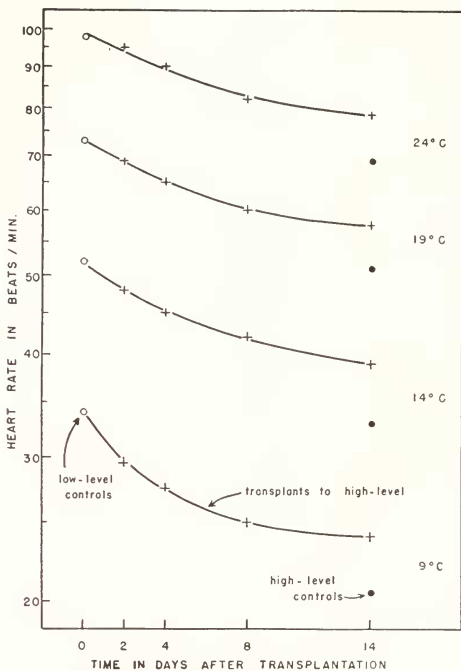


FIGURE 5. Acclimation of heart rate in low-level transplants to high-level 2, 4, 8, and 14 days after transplantation. Points represent equal weight (0.6 gm.) animals taken from the weight regression curve of each sample. Low-level controls measured at beginning of experiment, high-level controls at end and low-level transplants to high-level at days indicated.

It is of interest to notice that there is a proportionately greater change in heart frequency of the transplants at 9° C. than at any of the higher temperatures up to 29° C. This differential response may have as a basis the difference in slope of the rate/temperature curves of high- and low-level controls. The Q_{10} 's of the heart rate show the low-level or cold acclimated group to be less sensitive to temperature change at lower temperatures, i.e., the curve is flatter between 9° and 14° C. An average 0.6-gm. animal from low-level has a Q_{10} of 2.33, while that of equal weight

TABLE III

Calculation of per cent acclimation 14 days after transplantation from low- to high-level and calculation of Q_{10} at several temperatures, 2, 4, 8, and 14 days after transplantation. Heart rates obtained as in Table II. Per cent acclimation calculated as in Table II. Performed February 1954.

Temp. ° C.	Heart rates of average 0.6 gm. animals in beats/min.						Acclimation after 14 days %	
	High cont. 2/1/54	Low cont. 2/1/54	Trans. to high. Days after trans.					High cont. 2/15/54
			2	4	8	14		
9	20.0	34.0	29.5	27.5	25.0	24.0	20.5	74.0
14	32.0	52.0	48.0	45.0	42.0	39.0	33.0	68.5
19	51.0	73.0	69.0	65.0	60.0	57.5	51.0	70.5
24	67.0	98.0	95.0	90.0	82.0	78.0	69.0	67.5

Q ₁₀ values from these figures							
9-14	2.56	2.34	2.65	2.68	2.80	2.64	2.67
14-19	2.54	1.97	2.07	2.08	2.04	2.17	2.38
19-24	1.77	1.80	1.90	1.92	1.87	1.86	1.83

high-level and transplant to high-level is 2.70 and 2.69, respectively, after 14 days. The difference in Q_{10} decreases with increasing temperature. The change in slope of the rate/temperature curve requires a proportionately greater change in heart frequency at lower temperatures.

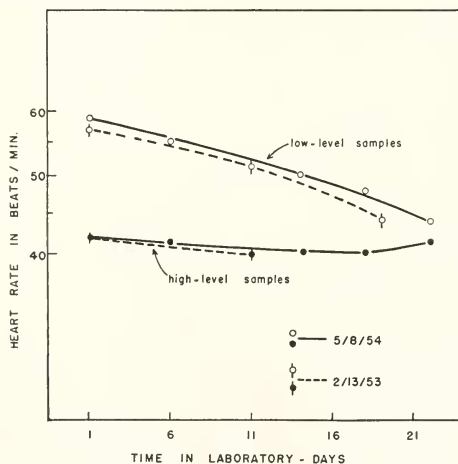


FIGURE 6. Heart rate during laboratory acclimation. High- and low-level samples kept cool (14° C.) and without food. Samples taken on days indicated; heart rate recorded at acclimation temperature (14° C.). Points represent equal weight (0.5 gm.) "average" animals taken from weight regression curves.

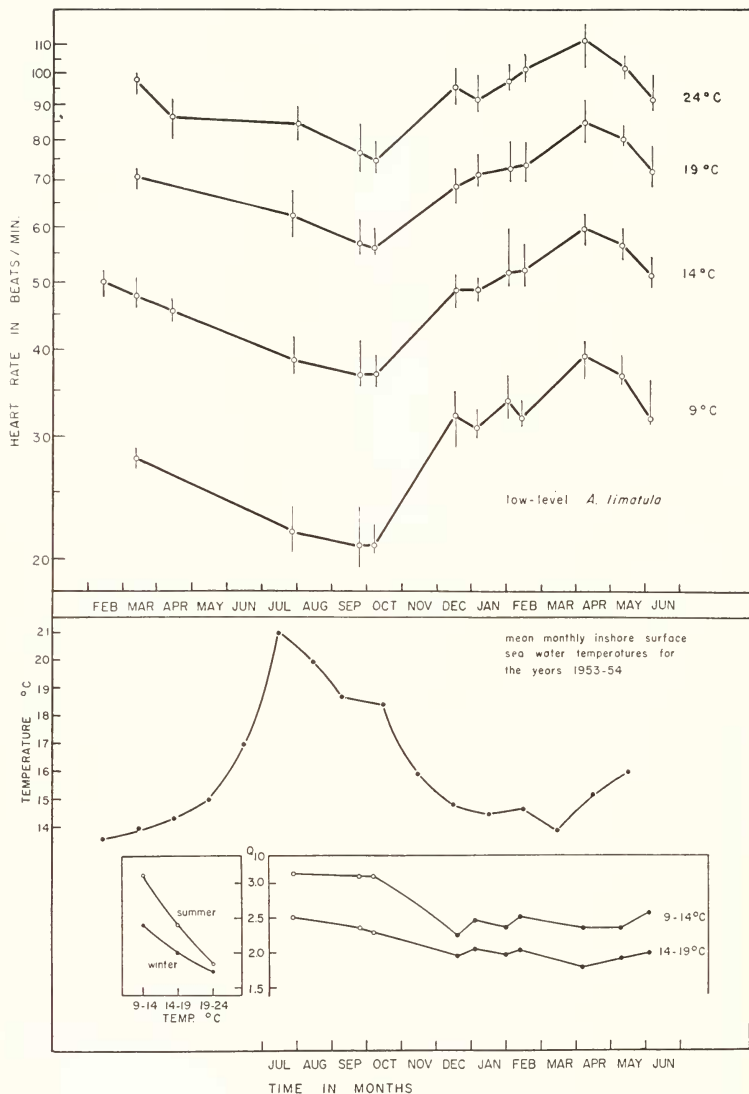


FIGURE 7.

Fourteen-days: time course. In early February, 1954, 100 low-level limpets were moved to high-level at site 4. A total of 60% were recovered. Of 50 low- and 50 high-level controls, 50% and 60%, respectively, were recovered. Transplants were collected 2, 4, 8, and 14 days after transplantation.

Within two days a decrease in the heart rate of transposed individuals is noticeable. The further decrease in heart rate is non-linear plotted semilogarithmically, declining most rapidly in the first 8 days (Fig. 5).

Roughly 70% of the difference in heart rate was achieved by 14 days (Table III). This figure is arrived at by using the high-level control values (at 14 days) as a criterion of complete acclimation. However, for the reason given above (see second experimental transplantation), 70% is too low a figure.

The response of the transplants to short term temperature steps is remarkably similar in both this and the previous experiment. Not only is there a proportionately greater decrease in heart frequency at 9° than at 24° C. after 14 days, but the differential response is apparent after two days. Table III shows the calculated Q_{10} 's of the heart rate for equal weight controls and transplants. The change in slope at the lower end of the rate/temperature curve is conspicuous within two days.

Behavioral Response to Transplantation

On all three occasions, when low-level limpets were transposed to the level of high-water individuals, it was observed that the transplants moved from the site of placement.

In the first group of low- to high-level transplants, recovered on April 11, 1953, 29 days after transplantation, all surviving animals were found buried beneath a shell and gravel deposit at depths to 6 inches. High-level controls were recovered from the basalt outcroppings where they were naturally located, whereas the transplants, originally placed in close proximity to the controls, had migrated vertically downward from 1-6 inches out of the direct sunlight and into the damp deposit. A return to their old level is considered to be impossible on the boulder strewn beach.

The identical behavioral response was elicited from transplants in subsequent experiments. All surviving animals were found to have moved either vertically downward or horizontally under overhanging rocks, in both cases into cooler and damper regions. During the closely watched experiment, migratory movements were found to be complete two days after placement. High-level individuals, again, remained where they had been placed, in some cases in the identical spot.

The behavioral response to transplantation has bearing upon the heart frequency relations and is discussed in connection with those measurements.

FIGURE 7. Heart rate as a function of season for low-level *A. limatula*. Upper four curves—horizontal lines connect points for equal weight (0.6 gm.) animals taken from weight regression curves. Vertical lines denote total variation around regression lines within the weight range of 0.4-0.8 gm. The weight selected for comparison is from the middle of the usual range of weight at any collection period and from the comparable linear segment of the regression curves. Fifth curve—mean monthly inshore surface water temperatures in °C. from Redondo Beach, California (area adjacent to northernmost collection site) for the years 1953-1954. Points are monthly means calculated from the values of four daily recordings made between 9:00 A.M. and 6:00 P.M. Lower two curves— Q_{10} values calculated from the data above (upper 4 curves) for the temperature intervals shown. Inset— Q_{10} plotted against temperature to compare summer and winter averages.

Laboratory Studies

Additional, though indirect, laboratory evidence substantiates the field studies on acclimation. High- and low-level *A. limatula* were maintained at 14° C. in the laboratory without food for periods up to 22 days. Five to 10 individuals were withdrawn, one day after collection and at intervals thereafter, and the heart beat counted at 14° C. The heart rate of an average weight low-level animal, selected from the weight regression curves, decreased 25% of the initial rate after 22 days. A similarly obtained equal weight animal from high-level showed no appreciable decrease in rate in the same number of days (Fig. 6).

At the time of collection, low-level animals were living in the field at approximately the temperature of the experiment. The decrease in heart rate is presumed to be due to starvation uncomplicated by a tendency to acclimate. High-level animals, on the other hand, were living in the laboratory at a temperature lower than that in the field. At 14° C. two opposing forces are at work on these animals: starvation tending to decrease the heart rate and acclimation to a lower temperature tending to increase it.

Effect of Season on Heart Rate

Low-level population. Low-level *A. limatula*, collected during the winter and spring, have faster heart rates at all temperatures from 4° to 29° C. than animals of equal weight collected in summer (Fig. 7). The seasonal trend in heart frequency is in good agreement with the change in the mean monthly surface water temperature. In general, there is an inverse relationship such that an increase in temperature is followed by a decrease in heart rate which increases again with waning temperatures. The difference in winter and spring recordings of 1953 and 1954 has a basis in the habitat differences of the samples taken during those periods. Animals were collected from different sites during the comparable periods of each year. Low-level animals collected in 1953 were apparently slightly more subject to warming than were those animals collected in 1954.

The mean absolute change in rate (from winter to summer) at 24° C. is greater than at lower temperatures but the proportionate change at 9° C. exceeds that at higher temperatures. At 9° C. there is a maximum 46% change in rate as compared with 29% at 24° C.

Temperature sensitivity of the heart rate (as measured by the Q_{10}) similarly changes with season (see Fig. 7 and Table I). Winter and spring animals show lower Q_{10} 's and thus decreased sensitivity to temperature change between 9° and 24° C. With increasing temperature the difference in Q_{10} between winter and summer animals decreases. There is no appreciable difference if we compare Q_{10} 's at temperatures at which winter and summer animals show equal rates (at 14° C. or above for winter animals).

High-level population. When the seasonal change in heart rate of high-level forms is examined, the picture is less clear than that obtained for low-level forms (Fig. 8). There is a suggestion of the inverse relationship with seasonal temperature change, but the range in heart frequency is smaller and a response to short term temperature fluctuations, of the order of several days to a week, is evident (see February, 1954, Fig. 8). Although high-level animals were always chosen from the upper extreme of the intertidal range of the species, the possibility of habitat differences between samples cannot be ruled out.

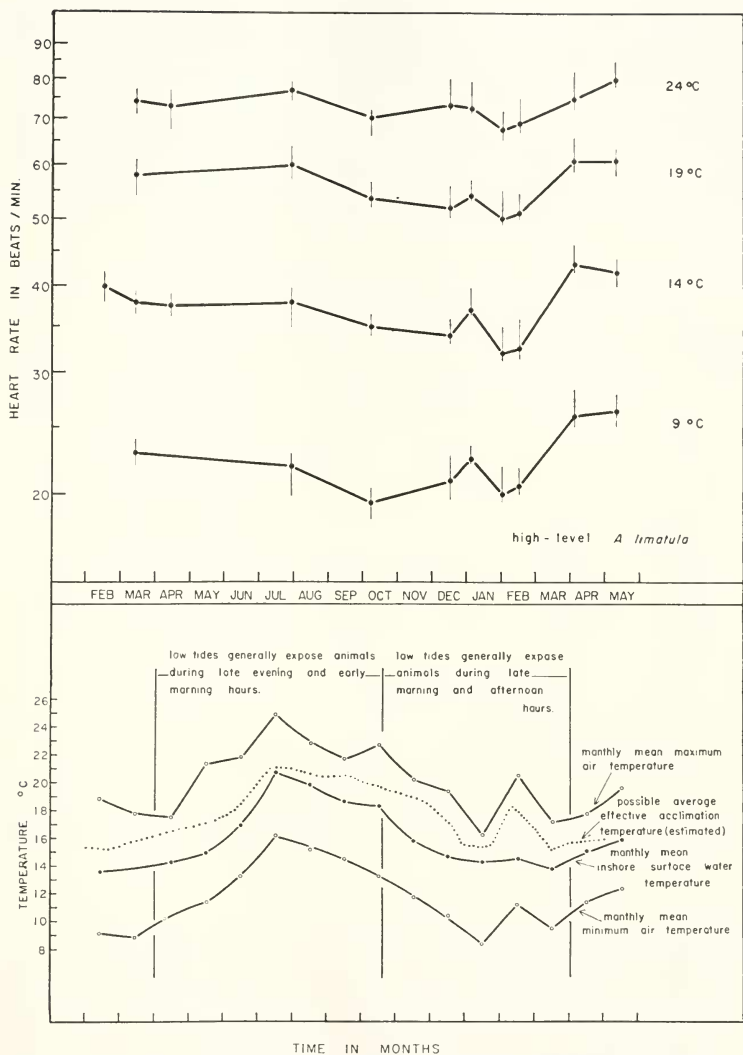


FIGURE 8. Heart rate as a function of season for high-level *A. limatula*. Parameters of upper 4 curves are the same as in Figure 7. Lower series of curves show (1) mean maximum and minimum monthly air temperatures (—○—) taken 4 times daily at Santa Monica, California; (2) monthly mean inshore surface water temperatures (—●—) as in Figure 7 but on reduced scale; and (3) possible average effective acclimation temperature (estimated) based on the heart rate response (· · · · ·).

High-level animals begin pre-spawning buildup of gonadal tissue in July and appear to maintain ripe gonads through October (see section on gonad size). The rather rapid heart rates in late July and possibly also in early October may be due to an increased metabolic activity associated with gametogenesis.

Table I shows the Q_{10} values calculated from the curves in Figure 8. Samples taken during "characteristic" winter months (January, April) show decreased temperature sensitivity (lower Q_{10} 's) than samples taken in "characteristic" summer months (August). However there is a marked lack of consistency in the temperature sensitivity of the heart rates of high-level animals. Samples taken during

TABLE IV
Gonad size as a function of season in high- and low-level A. limatula

Date of collection	Average gonad size in % wet weight of soft parts					
	High level	Gonads turgid	No. of specimens	Low level	Gonads turgid	No. of specimens
2/13/53	8.6		20	29.3		26
3/13/53	7.6		14	16.3		12
4/11/53	0.0		22	16.1		21
7/12/53	—	X	18	—	X	18
7/27/53	21.8	X	14	20.2*		7
9/25/53	28.1†	X	6	—**		12
10/ 7/53	29.4		12	17.1		15
12/17/53	6.6		13	21.7		19
1/ 4/54	7.6		20	31.1		12
2/ 1/54	0.0		16	20.4		15
2/15/54	0.0		12	15.9		10
4/ 6/54	0.0		14	17.0		18
5/ 8/54	0.0		8	15.6		11
6/12/54	15.6		14	16.1		12

* Six additional animals spawned in field—weights not taken.

** All animals spawned in field—weights not taken.

† Five additional animals spawned in laboratory—weights not taken.

months of unseasonal temperature fluctuations (February, 1954) do not show the Q_{10} associated with adaptation of the heart rate to a warmer temperature, although the rates themselves have adapted.

Gonad Size

In relation to intertidal height. Gonad size, as per cent of the wet weight of soft parts, has not been found to vary systematically with the size of the organism over the range of 0.3–1.2 gm. Therefore, the mean gonad weight, as per cent of the body weight, of the animals of each collection period was calculated. These values are presented in Table IV.

Excluding the period roughly from early June to mid-October, low-level *A. limatula* appeared to maintain a larger gonad than high-level forms of the species. Mean gonad weight of low-level individuals did not fall below 15% of the body weight dur-

ing the 1½-year period of observation. High-level individuals, from approximately November until June, possessed either small or negligible amounts of gonadal tissue with as many as 60% showing indeterminate sex. Indeterminate sex among low-level individuals was observed during the month of September after spawning occurred in the field.

In relation to season. Natural spawning among high- and low-level individuals does not appear to occur at similar times. Turgidity of the gonad (regardless of size) and deformation of the female gametes have been used to indicate the presence

TABLE V
Effect of transplantation on size of gonad in A. limatula

Date of collection	Average gonad size in % wet weight of soft parts							
	Controls				Transplants			
	High level	No.	Low level	No.	Low to high	No.	High to low	No.
Experiment I—29 days								
3/13/53	7.6	14	16.3	12				
4/11/53	0.0	22	16.1	21	0.0	18	15.9	20
Experiment II—14 days								
12/17/53	6.6	13	21.7	19				
1/ 4/54	7.6	20	31.1	13	21.9	10		
Experiment III—at intervals to 14 days								
2/ 1/54	0.0	16	20.4	15				
2/ 3/54					19.9	9		
2/ 5/54					21.4	9		
2/ 9/54					21.2*	4		
2/15/54	0.0	12	15.9	10	7.3**	4		

* Six other animals spawned in laboratory.

** Six other animals without weighable gonads at time of collection.

of pre-spawning ripeness—in agreement with Fritchman (1953). In July, samples from both high- and low-level revealed this condition (see Table IV). Later in the same month, partial or complete spawning of 50% of the low-level sample had occurred. Spawning of the entire low-level population is assumed to have taken place before late September.

High-level individuals, on the other hand, showed a persistent turgidity of the gonads throughout late September in the field, although under maintained temperature (17°) in the laboratory, 50% of the September sample spawned. In early October, weight of the gonads of high-level forms had not significantly changed, but turgidity was no longer apparent. By December, 50% of the high-level sample

was devoid of weighable gonadal tissue; by February, 100% of the animals showed this condition and remained so until the following June. After September, spawning among the low-level population was apparently at an end; the gonads then returned to approximately the average weight for non-spawning months. However, a second buildup of the gonads occurred in midwinter (January and February of two consecutive years) which, though of substantial weight, did not show the characteristic pre-spawning turgidity.

Effect of transplantation. Gonads of transposed animals were examined subsequent to recording of the heart rate. Data are available from three experimental transplantations and have been summarized in Table V.

1. After 29 days, in the spring, a complete reversal of the gonad size was obtained. High-level transplants to low-level developed gonads whose average size was not significantly different from that of the low-level controls.

2. During January, 1954, the second gonadal buildup occurred among low-level individuals. While the average gonadal weight of low-level controls increased by approximately 50% in 14 days, that of the transplants from low- to high-level did not increase; rather they averaged the same size as before transplantation.

3. During February, 1954, animals were collected 2, 4, 8, and 14 days after transplantation from low- to high-level. No change was observed in the size of the gonads up to 8 days after transplantation although 60% of the individuals from the eighth day of collection spawned in the laboratory (19° C.). At the end of 14 days, 60% of the transplanted individuals possessed negligible gonads and the weights of the remaining 40% averaged less than one-half that of the low-level controls on the same date.

DISCUSSION

Microgeographic variation. The preceding data show that the microgeographic intertidal distribution of a gastropod, *Acmaca limatula*, is reflected in certain physiological and morphological differences (see Segal, Rao and James, 1953, preliminary report). The differences found in relation to shell and body size have been reported previously (Segal, 1956). High intertidal *A. limatula* are found about one meter above zero datum; low intertidal *A. limatula* are found at zero datum and below. High-level forms show a slower heart rate than low-level forms when both are measured at any given temperature from 7° to 29° C. Comparisons show that high intertidal animals are exposed about 50% of the time and are subjected to air temperatures which rise above and fall below that of the ocean. Low intertidal animals are submerged over 90% of the time and live essentially at the temperature of the ocean.

We do not have the complete curve of temperature against time for the high-level animals; therefore, we do not know how the temperatures they are acclimated to are related to the temperature fluctuations they are subjected to. We only know that these animals respond as if they are living at a higher temperature than that of the ocean. In this regard, Kirberger (1953) maintained an annelid, *Lumbriculus variegatus*, for 12 hours alternately at 16° and 23° C. for 8 to 14 days. She compared the O₂ consumption of these animals with that of two groups kept solely at 16° and at 23° C. for the same period of time. Those kept at the alternating tempera-

tures averaged the fluctuations and responded as if they were adapted to 19° C. Animals maintained at the constant temperatures showed the typical compensatory response, *i.e.*, those animals from 16° C. consumed more O₂ than those from 23° C. when measured at the same temperatures.

Numerous studies have shown similar physiological differences to exist between macrogeographically distributed populations of a species. The question has been raised as to whether these animals are, in fact, members of the same species. Such latitudinal studies where the physiological differences are clearly correlated with habitat temperatures (Mayer, 1914; Spärck, 1936; Fox and Wingfield, 1937; Fox, 1939; Roberts, 1952; Dehnel, 1955) are sufficiently similar to intertidal microgeographic studies to warrant the suggestion that the same compensatory phenomenon is involved. High intertidal individuals, similar to warm seas populations, behave as though they are warm-adapted relative to low intertidal individuals and cold seas populations. Rao (1953), and Segal, Rao and James (1953), in the only studies where microgeographic and macrogeographic physiological differences have been compared in the same species (*Mytilus californianus*), show that 2½ feet of vertical separation is equivalent to about 350 miles in latitudinal separation. The rate of water propulsion in low-level northern mussels differs as much from that in low-level southern mussels as the rate in low-level southern mussels differs from that in high-level southern mussels 2½ feet higher in the intertidal zone. The data suggest that we are dealing with the phenomenon of individual adjustment to habitat temperatures; in short we may hypothesize that this is a phenotypic adaptation. In the present study we have made a more direct test of this hypothesis.

If a physiological rate character is, in time, readily reversible under changed temperature conditions, we may say that this rate attribute is acquired during the ontogeny of the individual. These changed temperature conditions may be artificially imposed by laboratory acclimation or by transplantation of the organism in the field; they may be naturally imposed by the changing season.

When low-level *A. limatula* were transposed to high-level, slowing of the heart rate was evident in two days and full adaptation was accomplished within 14 to 29 days. The reverse, adaptation to cold, was also complete within 14 to 29 days (Figs. 3, 4, and 5). Thus, the difference in heart rate of individuals at different intertidal levels was shown to be reversible under habitat conditions.

There are few published reports on transplanting individuals of a species from one habitat to another, using some physiological rate character as a measure of adjustment. Sumner and Lanham (1942) and Loosanoff and Nomejko (1951) report instances of transplantation with no apparent acclimation. These results may be due either to the inability of a homogeneous species to acclimate as in the first reference cited, or to the existence of true physiological races as in the second reference. Physiological races have been demonstrated previously both among field and laboratory populations (Brown, 1929; Goldschmidt, 1932, 1934; Hovanitz, 1947; Stauber, 1950). The transplantation method seems to be effective for revealing the nature of intraspecific physiological and morphological differentiation (Moore, 1934; Segal, 1956).

Acclimation of the heart rate has also been shown to occur with the seasonal change in temperature. Low-level animals have about the same rate in winter and in summer at their respective field temperatures (Fig. 7). High-level animals

show responses to unseasonal air temperature fluctuations which tend to mask the seasonal acclimation (Fig. 8). In substantiation of the field studies, acclimation to cold has also been demonstrated in the laboratory (Fig. 6).

Temperature sensitivity. The sensitivity of the heart rate to temperature change, measured by Q_{10} , has also been shown to vary with intertidal height. Between 9° and 19° C., but not as clearly above 19° C., low-level, cold adapted organisms show lower Q_{10} 's than equal weight high-level organisms (the rate of change over temperature intervals is used rather than at temperature points because it is believed that rates of 1° C. increments are necessary for a reliable estimation of the change in rate at a given temperature). Bělehrádek (1935), using examples taken from data of various investigators, points out that temperature coefficients commonly increase with the adaptation of the protoplasm to higher temperatures. This thesis is further strengthened through additional evidence of Rao (1953) and recalculations by Rao and Bullock (1954) of earlier equivocal data.

Of interest are the temperature relations of high and low intertidal groups. Although low-level animals respond as though they are cold acclimated relative to high-level animals, environmental temperatures below 13° C. are probably rarely encountered in this area. High-level animals do meet with such temperatures during the winter and spring months when the higher of the two low tides and the lower of the two high tides of each day are of insufficient magnitude to cover the animals. It is worthy of note that on the two occasions when heart rates were measured at 7° C. (at 4° C. both groups show cold depression and cessation of beat in a fair percentage of each sample), low-level animals show higher Q_{10} 's between 7° and 9° C. It indicates that the low-level, cold acclimated group is paradoxically approaching cold depression at a higher temperature than the relatively warm acclimated, high-level group. It further suggests that the physiological temperature range (that range of temperatures over which there is no observable indication of depression) extends farther into the cold in the warm acclimated group. Above 29° C. heart beats could no longer be counted with accuracy, but the very fact that high-level animals have been found with higher body temperatures in the field, while the surface ocean temperature rarely if ever has exceeded 24° C. in this locale, permits the interpretation that the physiological range similarly extends farther into the warm. Dehnel (1955) reaches the same conclusion for optimal temperature range of larval growth within the species in populations from Southern California and Alaskan waters.

Additional confirmation exists for the thesis that cold-adapted organisms show lower Q_{10} 's and thus greater independence to temperature change. Winter animals as compared with summer animals have lower Q_{10} 's, at least from 9° to 24° C., and animals transplanted from low-level (cold) to high-level (warm) show an increased temperature dependence (higher Q_{10} 's) within two days.

Scholander *et al.* (1953), in a metabolic study of arctic and tropic poikilotherms, suggests that a low Q_{10} would only be advantageous to offset the effects of changes in temperature due to diurnal, seasonal, or migratory factors. The authors state that no such adaptation was found among the species which would profit from a low Q_{10} : temperate water forms, fresh water forms, and terrestrial forms. Rao and Bullock (1954) agree that at present no general case can be made for lower Q_{10} 's in forms exposed to changing temperature but argue that cold adapted, *e.g.*, arctic

species as compared with tropic species, do show lower Q_{10} 's even though they may not be normally exposed to changes in temperature.

The present study is unique in that comparisons are made between animals which are living under fluctuating temperature conditions (high-level, exposed) and animals which are living under relatively constant temperature conditions (low-level, submerged). In this locale the high-level forms are exposed to considerably higher temperatures than that of the ocean and these animals act as though warm adapted. As shown, the warm adapted animals have the higher Q_{10} in spite of the fluctuating temperature of the habitat. Similarly, the summer forms from both high- and low-level have higher Q_{10} 's.

These differences could not have been expected simply from measurements of the Q_{10} of the *species* at any one time. If individuals of a species residing in different microhabitats and from one season to another show variations in temperature sensitivity, a great burden is placed upon comparisons between species. Other than for species living in arctic and tropical seas, with their almost constant temperatures, it is doubtful whether Q_{10} values (or any temperature coefficient describing sensitivity to changes in temperature) of a physiological rate activity are meaningful except in very limited comparisons. The thermal history of one segment of a species is not the thermal history of that species. The range of Q_{10} values (at a given temperature) permissible within the genetic makeup of a species would describe the temperature sensitivity of that species.

Spawning and gonad size. If the data on spawning represent normal behavior, then low-level *A. limatula* spawn before high-level *A. limatula*. Now the question remains as to whether high-level *A. limatula* actually spawn. All low-level animals show partial or complete spawning by late August. High-level animals, on the other hand, if they do spawn, do so sometime between October and December. We are not sure that high-level animals spawn because gonadal turgor and deformation of the female gametes, which Fritchman (1953) considers as indicative of pre-spawning ripeness, were not present in October although the gonads of high-level animals were of large size. Therefore, we must assume either that (1) high-level animals do not spawn in the field, or (2) pre-spawning ripeness is not a necessary condition. Again, if spawning occurs, it is out of phase with that of the low-level population. Yet, Fritchman (1953), working with high- and low-level members of two species of the same genus in central California (*A. fenestrata cribraria* and *A. testudinalis scutum*), did not find a difference in spawning time.

With the warming of the ocean in May and June (Fig. 8), gametogenesis is stimulated in both high- and low-level populations. By July, all animals showed the gonadal turgor and deformation of the female gametes associated with the pre-spawning ripe condition. Spawning occurs in the low-level population; this population is submerged and therefore subjected to the more constant temperatures of the ocean. High-level animals, which are only submerged 50% of the time, do not spawn during this period of warmest average sea water temperatures (July, August, September: 21°–19° C.). But, by October, the characteristics associated with pre-spawning ripeness have disappeared although the gonads are still large. From October to December, when the average inshore surface water temperatures have fallen to 17° C. and below, the high-level animals lose their gonads. The trigger mechanism necessary to initiate spawning may well be a required time interval spent

at a given temperature rather than the reaching or exceeding of that temperature for a short interval of time. Fifty per cent of the high-level animals (September collection; mean ocean temperature 19° C.) spawned in the laboratory after three days at 17° C.

The loss of the gonad sometime between October and December coincides with the seasonal tidal change; from October through April high-level animals are exposed during the late morning and afternoon hours (Fig. 8). It is during these hours that these animals are subjected to direct solar radiation and to heat conduction from the exposed rock substratum. The presence of a large gonad, or for that matter any gonadal material, would decrease the area under the shell available for water and thereby decrease the animals' ability to avoid desiccation (Segal, 1956). Low-level animals transplanted to high-level lost the gonadal material within two weeks (Table V). The evidence suggests that we are dealing with a non-breeding population living at the extreme of the intertidal distribution of the species.

SUMMARY

1. Highest and lowest members of a eurytopic intertidal species, *A. limatula*, have been compared in the following: heart rate, gonad size, and spawning behavior.

2. Within the weight range of 0.4 to 1.0 gm., the heart rate varies inversely with increasing weight. The regression coefficients fall between -0.042 and -0.172 ; thus no single expression is available to describe fully the effect of weight on heart rate in this species.

3. Sex and size of gonad (as divorced from turgidity) have not been found to contribute to the variation in heart rate between samples.

4. Comparing equal weight animals, it is found that low intertidal individuals have faster heart rates than high intertidal individuals at any temperature from 4° to 29° C.

5. From data on field temperatures it is suggested that the significant parameter of the intertidal difference is temperature. High-level animals are subjected to considerable periods of warmer as well as to some periods of cooler temperatures than are low-level animals.

6. An attempt was made to characterize the difference in heart rate by: transplanting the animals in the field, following the seasonal changes, and maintaining samples of both populations in the laboratory at a cool temperature (14° C.) and without food.

7. When low-level animals are transplanted to high-level their heart rates slow so that within 29 days it is equal to that of the high-level animals when measured at any given temperature. The half-acclimation time was about 6 days. In the field, acclimation to cold was also shown to be complete within 29 days.

8. Comparisons of the heart rate during winter and summer showed that both high and low intertidal animals have faster rates in winter at any given temperature from 9° to 29° C. Acclimation to cold was also shown in the laboratory.

9. The above results lead to the interpretation that the microgeographic difference in heart rate is a phenotypic expression of a compensatory phenomenon operating to maintain approximately equal heart activity in spite of the habitat temperature differences. Latitudinal differences in physiological rate activities which

are clearly correlated with habitat temperature are sufficiently similar to the intertidal differences reported here to warrant the suggestion that the same phenomenon is involved.

10. Low-level and winter animals show a heart rate that is less dependent on temperature changes in the range from 9° to 19° C. This same response is not observed consistently above 19° C. While both high- and low-level animals appear to be approaching cold depression below 9° C., the low-level animals are cold depressed at a higher temperature than the relatively warm-adapted high-level animals; the low-level animals possess a higher Q_{10} in this range. It is suggested that the physiological temperature range of the warm-acclimated group extends both higher and lower than that of the cold-acclimated group. In the field, the Q_{10} of the heart rate changes within two days after the animals are transplanted.

11. The size of the gonad also varies with intertidal height. Low-level animals maintain a larger gonad during winter and spring than do high-level animals. Transplantation also reveals this difference to be reversible.

12. Analysis of spawning behavior (using turgidity as the criterion of pre-spawning readiness) presents the possibility that either (1) the two groups spawn a number of months out of phase with each other, or (2) high-level individuals do not contribute to the breeding population.

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